ON THE DISTRIBUTION OF ANTIBODIES AND THEIR FORMATION BY THE BLOOD.*

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IN order to study the distribution of antibodies in immunized animals, as well as certain other questions, dogs were injected intravenously with the blood of white rats or of goats. Only one injection was made, the amount injected being 1 c.c. of a 10 per cent suspension of the whole blood per kilo of dog. The suspension was made in m/8 NaCl solution containing one per cent of sodium citrate. The animals so injected were killed at different intervals after the injection and the amounts of antibodies in the blood, lymph, etc., determined. In this way estimations were secured of the antibody content in the fluids in question at different periods of immunization.

Collection of the fluids and methods of testing.—The lymph and aqueous humor were collected under ether anesthesia. The aqueous humor was drawn into a pipette forced through the cornea. Sterile glass cannulae were inserted in the neck lymphatics and the thoracic duct, and the lymph allowed to flow into sterile tubes. In the case of the neck lymph gentle massage of the head and neck was usually employed to hasten the flow. The blood was collected at the end of the collection of lymph. The cerebrospinal fluid was obtained through an opening into the fourth ventricle after the animal had been bled to death, this guarding against admixture with blood. The neck lymph always appears to be free from red corpuscles except when dissection is made cephalad to the point of insertion of the cannula in the lymphatic and in case of tearing of the skin and injury to the deeper parts of the head and neck as in fighting. The thoracic lymph may contain some erythrocytes even when there is no evidence of injury to the viscera, the trunk, or the legs. Perhaps the struggling of the animal during the anesthetization is responsible for the escape of red corpuscles into the thoracic lymph. In all cases the fluids from each animal, or set of animals, were treated in exactly the same manner; the blood and lymph were allowed to clot and the sera withdrawn after 18–24 hours; the fluids were tested at the same time under the same conditions and on the same objects. If it were necessary to keep the fluids for some time before testing them they were placed in a cold chamber at -1 or $-2°$ C. In almost every case the same identical experiment was made on two dogs of about the same size and weight which were kept under the same conditions. In the case of the dogs injected with rat blood all determinations were made with fresh, unheated fluids. In the case of the dogs injected with goat blood the lysin and opsonin determinations were made with unheated fluids. On account of prompt lysis of goat corpuscles by the lower dilutions

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of some of the fluids it was necessary to heat the fluids to 58° C. for 30 minutes for the agglutinin determinations. It was found that the maximum lysis by the antigoat fluids is obtained by the addition in each case of 0.012 c.c. fresh guinea-pig serum which in that quantity is without lytic power by itself. In Table 1, giving the results in question under "Lysin," the figures below the short line represent the highest dilution of unheated serum of fluid giving distinct lysis without the addition of 0.012 c.c. of guinea-pig serum, while the figures above the line give the highest dilution causing lysis with the addition of 0.012 c.c. of fresh guinea-pig serum.

In making these determinations the total quantity in each mixture was 0.6 c.c. Every mixture contained 0.2 c.c. of a five per cent suspension of washed, fresh corpuscles, goat or rat as the case might be; in the phagocytic experiments the remainder was made up of 0.2 c.c. suspension of dog leukocytes (obtained from pleural aleuronat exudates) and the antifluid tested plus the requisite amount of m/8 NaCl solution; and in the lytic and agglutinating experiments the remainder was made up by the fluid tested, 0.12 c.c. guinea-pig serum in the case of one of the lytic tests with goat corpuscles, and sufficient NaCl solution. After the usual incubation periods the lytic and agglutinating mixtures were placed in the ice-box for 18–24 hours when the results were determined, while in the case of the opsonic tests smears were made after an incubation of 60 minutes, and then stained. The results are given in Tables 1 and 2, the figures of which indicate the highest dilutions at which undoubted antibody action was observed on part of the fluids there mentioned. On Charts 1, 2, 3, 4, and 5 some of the results of the estimations of antibody content in the fluids of one dog of each set are plotted out in form of curves, the abscissae marking the day after injection on which the animals were killed and the fluids obtained, while the ordinates indicate the limits of antibody action. The results charted are those indicated by bold-faced type in Tables 1 and 2.

DISTRIBUTION OF SPECIFIC ANTIBODIES IN DOGS INJECTED WITH GOAT BLOOD.

The results shown in Table 1 and Charts 1, 2, and 3 indicate clearly that so far as the blood, the lymph from the thoracic duct, and the neck lymph are concerned, the changes in the concentration of antibodies during the course of active immunization run practically parallel. The concentration in the two lymphs is nearly the same, being on the whole a little less in the neck than the thoracic lymph, but in both it is always somewhat lower than in the blood-serum. In lymph as well as blood the antibody curves correspond quite accurately with the simple antibody curve obtained by determinations at short intervals of the content in antibody of the blood of the same animal. Indeed the general outlines of our composite curves correspond notably well with those of the simple antibody curve of blood-serum, especially when it is considered that the fluids from the different sets of animals were tested at different times, each time with

TABLE 1.

SPECIFIC ANTIBODIES IN BLOOD, LYMPH, AQUEOUS HUMOR, AND CEREBROSPINAL FLUID OF DOGS INJECTED WITH GOAT BLOOD.

LYSIN.

Days	Cerebrospinal Fluid		Aqueous Humor		Thoracic Lymph		Neck Lymph		Blood Serum	
1									48	..
	0	0	..	..	24	24	24	12	48	48
2	0	0	0	0	12		24	6	48	24
	0	0	0	0	12	...	6	3	24	12
4	0	0	0	0	384	192	384	96	1,536	768
	0	0	0	0	24	24	24	12	24	12
6	12	..	0		6,144	..	3,072	..	24,576	..
	0	0	..	..	12	24	3	12	24	24
9	0	6	24	48	3,072	3,072	384	384	12,288	12,288
	3	6	0	12	12	24	12	12	24	48
15	6	3	96	192	768	384	1,536	768	12,288	6,144
	0	0	12	0	24	24	12	12	48	48
20	0	3	6	6	48	72	192	192	768	1,536
	0	0	0	0	48	48	12	12	48	36
30	0	0	0	0	96	768	192	768	768	3,072
	0	0	0	0	24	24	24	24	48	48
40	..	0	..	16	....	384	....	288	....	768
		0		4		48		24		96

AGGLUTININ.

1	0	0	..	..	0	0	0	0	16	8
2	0	0	0	0	0	..	0	0	0	0
4	0	0	0	0	6	6	6	12	48	48
6	0	0	..	..	192	192	384	192	1,536	1,536
9	0	0	0	0	24	48	6	24	48	96
15	0	0	0	0	24	24	24	12	48	48
20	0	0	0	0	0	0	0	0	24	24
30	0	0	0	0	..	..	..	..	..	..
40	..	0	..	0	..	12	..	12	..	24

OPSONIN.

1	0	1	..	..	12	6	6	6	12	6
2	0	0	0	0	6	..	6	0	0	0
4	0	0	3	0	24	24	6	6	36	12
6	3	6	..	..	192	192	192	96	768	192
9	12	12	6	24	92	192	48	96	96	384
15	4	6	6	96	384	192	96	96	768	384
20	3	6	6	6	86	192	48	96	96	192
30	1	1	1	1	48	96	48	96	96	192
40	..	0	..	0	..	48	..	48	..	96

First day=normal dogs. Bold-faced figures=see Charts 1, 2, 3.

0=no action in dilutions of 1 to 1 or 1 to 3.

Under "Lysin" figures under short line give highest dilution with lytic power of the fluid in question without addition of guinea-pig serum; figures above the line represent highest dilution with lytic power in presence of 0.012 c.c. fresh guinea-pig serum.

a different suspension of goat corpuscles, and in the phagocytic experiments consequently also with different suspensions of leuko-

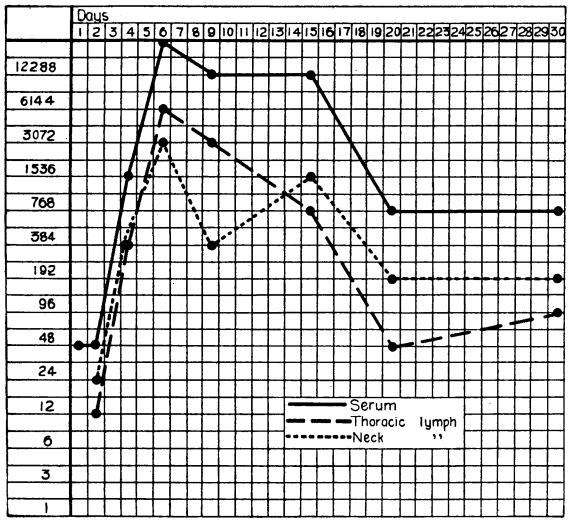

CHART 1.—Specific lysin in serum and lymph of dogs injected with goat blood.

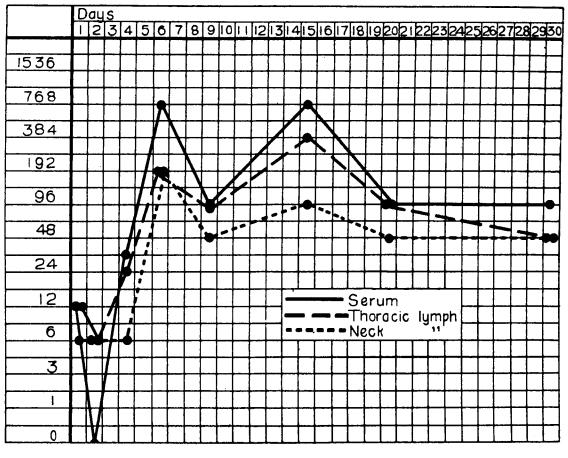

CHART 2.—Specific opsonin in serum and lymph of dogs injected with goat blood.

cytes as well. This last, together with variations in the power of individual animals to produce antibodies (compare the results obtained

in the two dogs of the same set, Table 1), no doubt in large measure accounts for the irregularities in the curves.

We may say, then, that in dogs injected with goat blood the newly formed antibodies in the lymph appear and disappear at the same

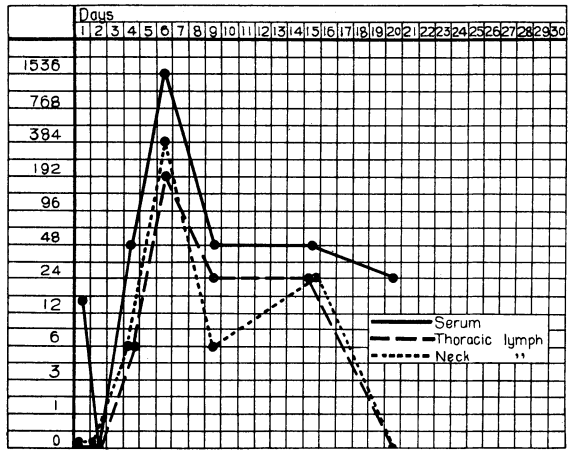

CHART 3.—Specific agglutinin in serum and lymph of dogs injected with goat blood.

time and describe the same wavelike curve as those in the blood. The relative concentration of the antibodies in the blood and in the lymph appears to be quite constant, but it would require further and more accurate measurements to determine the exact quantitative relations that obtain under various conditions.

As regards the cerebrospinal fluid and aqueous humor we note in the first place the complete absence in these fluids at any stage of the reaction of agglutinin. Lysin and opsonin, however, were present in both the fluids during the period of high antibody content in the blood and lymph (Table 1), but only in minute traces.

DISTRIBUTION OF SPECIFIC ANTIBODIES IN DOGS INJECTED WITH RAT BLOOD.

The results given in Table 2 and Charts 4 and 5 were all obtained with fresh serum. We note first of all the failure to obtain any evidence whatsoever of any increase in the lysin in the injected dogs. Thinking that perhaps owing to deficient or unsuitable complement

the absence of new lysin was merely apparent and not real, we made numerous trials with the fresh sera of the different laboratory animals as complement for antirat dog serum, and heated immune serum

TABLE 2.

SPECIFIC ANTIBODIES IN BLOOD, LYMPH, AND CEREBROSPINAL FLUID OF DOGS INJECTED WITH RAT BLOOD.

LYSIN.

Days	Cerebrospinal Fluid		Thoracic Lymph		Neck Lymph		Blood Serum	
1	0	0	6	6	6	6	12	12
2	0	0	3	0	3	0	12	12
3	0	0	1	1	1	1	12	12
6	0	0	6	6	3	3	12	12
9	0	0	6	12	0	3	12	12
12	0	0	12	6	0	3	12	12
16	0	0	6	..	1	1	12	12
20	0	0	9	9	3	3	12	12
30	0	..	6	..	24	..	12	..
44	0	..	6	..	6	..	12	..
AGGLUTININ.								
0	0	0	**48**	48	**48**	48	**96**	96
1	0	0	**3**	0	**3**	0	**24**	24
3	0	0	**12**	12	**24**	12	**80**	80
6	0	0	**192**	96	**192**	72	**384**	384
9	0	0	**384**	768	**384**	384	**3,072**	6,144
12	0	0	**384**	192	**384**	192	**1,536**	1,536
16	0	0	**192**	192	**192**	192	**1,536**	1,536
20	0	0	**768**	768	**768**	768	**1,536**	1,536
30	0	..	**384**	...	**192**	...	**1,536**	....
44	0	..	**72**	...	**0***	...	**192**	....
OPSONIN.								
1	**3**	3	**24**	24	**24**	24	**48**	48
2	3	3	**3**	3	**3**	3	**28**	24
3	0	0	**0**	24	**12**	24	**36**	36
6	6	12	**192**	192	**192**	192	**1,152**	1,152
9	48	48	**576**	768	**768**	768	**3,072**	6,144
12	192	192	**768**	768	**768**	768	**3,072**	3,072
16	24	6	**384**	384	**384**	384	**1,536**	768
20	12	12	**768**	768	**768**	768	**1,152**	1,152
30	12	..	**192**	...	**96**	...	**1,536**	....
44	12	..	**48**	...	**0***	...	**192**	....

* Lymph from hyperplastic goiter.

First day=normal dogs. 0=no action in dilutions of 1 to 1 or 1 to 3. Bold-faced figures=see charts 4 and 5.

plus fresh normal dog serum was used in varying proportions, but always with negative results. In the case of certain other dogs immunized with rat corpuscles a comparatively slight increase in lysin was observed, by use of either fresh serum or heated serum complemented with guinea-pig serum, while in a splenectomized dog

injected with rat corpuscles no increase in lysin was demonstrated.[1] It is possible, of course, that further search may reveal a method or

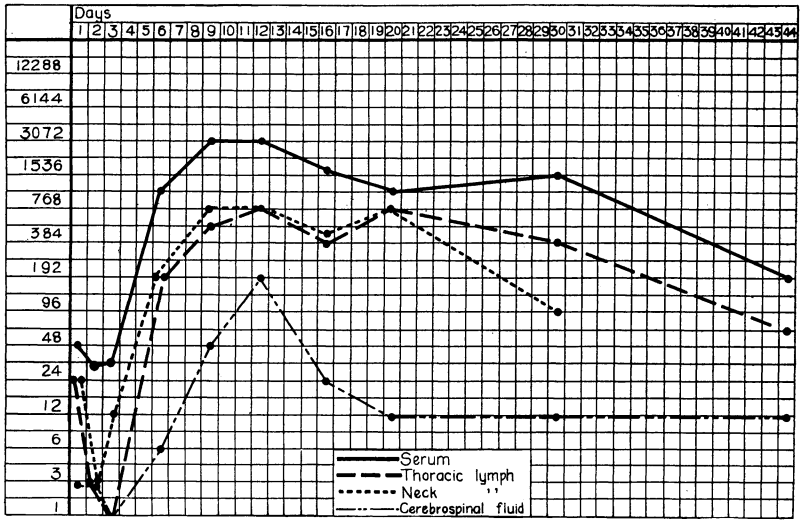

CHART 4.—Specific opsonin in blood, lymph, and cerebrospinal fluid of dogs injected with rat blood.

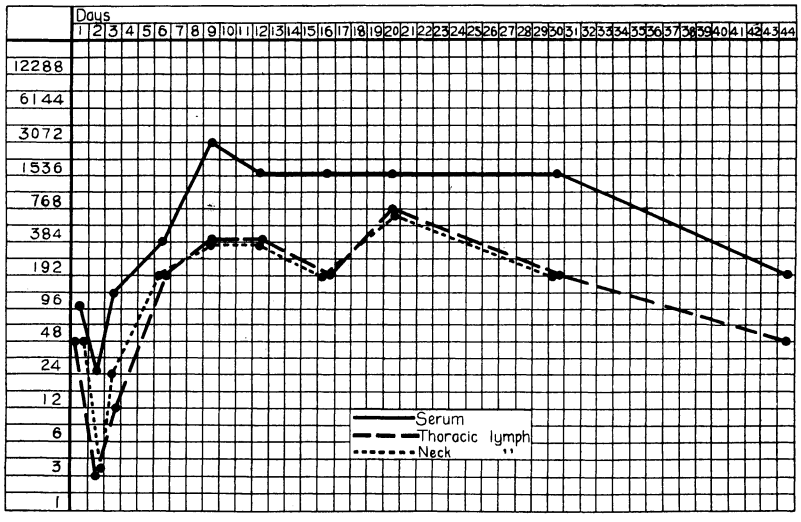

CHART 5.—Specific agglutinin in blood and lymph of dogs injected with rat blood.

methods that will bring to light an increase in lysin in dogs injected with rat corpuscles; the fact remains that with the methods we used

[1] Hektoen, "Opsonins Distinct from Other Antibodies," *Jour. Infect. Dis.*, 1909, 5, p. 78.

no increase was demonstrable in a single animal of the whole series. The agglutinin and the opsonin for rat corpuscles as compared with each other occur in about the same amount in the blood-serum, the thoracic lymph, and the lymph from the neck (Table 2 and Charts 4 and 5). Here again the blood-serum contains more antibodies than the two kinds of lymph; in the latter the concentration is about the same; the relative concentration in the blood-serum and the lymph is fairly constant, and the composite curves correspond closely with the normal or typical, simple antibody curve of the blood, a distinct negative phase being present in every curve. Finally, it is especially noteworthy that opsonin only was demonstrable in the cerebrospinal fluid in which it gives a fairly typical antibody curve; this would seem to indicate that this particular antibody finds an easy passage into the fluid from the blood and lymph, possibly with the water stream.

DISTRIBUTION OF THE SPECIFIC ANTIBODIES IN DOGS TRANSFUSED WITH THE BLOOD OF DOGS INJECTED WITH GOAT BLOOD.

In connection with certain other experiments it seemed desirable to investigate the occurrence and distribution of specific antibodies in dogs which had been transfused freely from dogs injected with goat blood. It was hoped that some light might be thrown on the question of the production of antibodies by leukocytes or other elements in the circulating blood and on the relative distribution of antibodies in the passively immunized animal.

As in the other experiments the immunization of the donors was effected by the intravenous injection of 1 c.c. of a 10 per cent suspension of goat blood per kilo of dog.

Transfusion.—In most of the transfusion experiments the donors were large dogs, weighing 22–28 K, and the recipients small animals, weighing 4–8 K. Hence one large dog would serve as donor for two of the smaller ones in transfusion from artery to artery and in cases of artery-vein transfusion, which was made in the usual way, one large dog would serve as donor for three small ones, because by the latter method the blood encounters no resistance in the recipient.

In the experiments involving the collection of lymph from the recipients, it was necessary to transfuse from artery to artery in order not to occlude any veins because venous occlusion and passive hyperemia quickly affect the character of the lymph from the region involved. With large dogs as donors and small dogs as receivers this result is attained by connecting the distal end of the carotid of the recipient with the

proximal end of the opposite carotid of the donor. This involves ligation of one carotid in the recipient which does not seriously alter the circulation in the neck and head. A "T" cannula of glass, coated on the inside with oil or paraffin, was used to connect the arteries of the two dogs. Theoretically a Crile cannula is superior to this device, but as thrombosis is equally well avoidable, the simple glass cannula was preferred because it may be manipulated more quickly. Placing the bulldog forceps on the carotids of both dogs proximally to the insertion of the cannula and carefully removing the blood in the free ends of the arteries before inserting the cannula prevents the blood from coming in contact with the cannula except during the transfusion and this contact does not start clotting. Donor and recipient, both under ether anesthesia, are placed on the back and the left carotid in one and the right carotid in the other is isolated and severed; the necks of the dogs are then brought closely enough together to permit the insertion of a "T" cannula in the proximal end of the carotid of the donor and in the distal end of the carotid of the recipient. The cannula is then filled with Ringer's solution and all air bubbles carefully removed. Everything being ready for the transfer of blood the recipient is bled "dry" from the proximal end of the severed carotid, that is, the carotid is left open until the flow of blood from it ceases; at this point no pulse is discernible anywhere, altho a feeble cardiac impulse may still be detected; the respiration is feeble, slow, and irregular, or it may have ceased. The clamp on the carotid of the donor is now removed and the donor pumps his blood into the empty vessels of the recipient until the pulse and blood pressure of the latter are brought up to the normal. In most of our experiments the transfusion was continued until the recipient's pressure was slightly higher than before the bleeding. The carotids of the recipient are now tied and the wound closed. The animals recover quickly from the anesthesia and in no case so far have any untoward symptoms ascribable to the exchange of blood developed.

Obviously the recipient loses the greater part of his own blood in this procedure and receives approximately an equal amount from the donor. Of course we are not dealing with exact quantities. The only criterion of the quantity of blood transferred from the donor is the blood pressure of the recipient which to a great extent depends on the condition of the vasomotor centers. It is probable that the normal activity of these centers is disturbed more or less by the temporary anemia of the nervous system and the collapse of the vessels. In nearly all our experiments the large dogs acting as donors were killed at the end of the transfusion. The collection of fluids and the estimation of antibodies were carried out in the manner already described.

The results of the experiments on the distribution of antibodies in the body fluids of dogs after bleeding and transfusion with immune blood may be disposed of quickly. As the figures in Table 3 show, the antibodies introduced in this manner very soon pass into the thoracic and neck lymph—one-half to three hours—and maintain about the same proportion in these fluids relative to that in the blood as in the actively immunized animals. The indications are that the antibodies appear in the thoracic lymph first, at least under certain circumstances, but further experiments with blood richer in anti-

bodies than used in our earliest determinations after transfusion are necessary to settle such questions. The results fail to show any notable difference in the rate of passage of the different antibodies from the blood to the lymph, but smaller differences might not be

TABLE 3.

ANTIBODIES IN FLUIDS OF DOGS TRANSFUSED WITH BLOOD OF DOGS INJECTED WITH GOAT BLOOD.

FLUIDS	HOURS AFTER TRANSFUSION WHEN FLUIDS WERE COLLECTED								
	30 Min.			3 Hours			24 Hours		
	Lysis	Aggl.	Ops.	Lysis	Aggl.	Ops.	Lysis	Aggl.	Ops.
Blood-serum	1,536	96	24	12,288	348	348	3,072	192	
Thoracic lymph	192	24	12	3,072	192	48	768	96	24
Neck lymph	12	0 (12)	0 (12)	768	24	12	384	48	12
Cerebrospinal fluid	3	0 (3)	0 (3)	12	0 (6)	0 (6)	6	0 (6)	0 (6)
Aqueous humor	12	3	0 (3)	24	6	8	0(6)	0 (6)	0 (6)

The figures in parentheses mean that there was no action in the dilution expressed by the figure.
Concentration of antibodies in blood-serum of donors at time of transfusion:

½ hour —lysis 6,144, agglutinin 384, opsonin 192
3 hours— " 24,576, " 1,536, " 1,536
24 hours— " 3,072, " 384, " 192

demonstrated by the method of dilution we used. Our figures indicate that in transfused animals traces of antibodies early may find their way into the cerebrospinal fluid and aqueous humor, but for unknown reasons this does not appear to take place constantly. Undoubtedly the rapid passage of antibodies from the blood into the lymph serves to explain to some extent the prompt early loss by blood in antibody in passive immunization.[1]

The fact that the relative distribution of antibodies on passive immunization is essentially the same as on active immunization indicates that the distribution in the latter case is determined by the rate of passage of antibodies from the blood to the lymph rather than by their place of formation. This conclusion seems well founded because the relative concentration is practically the same at all points of the immunization curves.

The number of instances in normal as well as immunized animals in which various antibodies have been found more concentrated in the blood than in the lymph and also more concentrated in the thoracic

[1] Levin, "Ueber passive Immunität," *Ztschr. f. Immunitätst. u. exp. Therapie*, 1909, 1, p. 3.

than in the neck lymph[1] makes it highly probable that this is the rule with respect to the concentration in these fluids of antibodies in general especially in the blood and lymph. In connection with this generalization it is interesting to note that Carlson and Luckhardt[2] find that the diastases in the normal body fluids are similarly distributed. It might be interesting to determine further the relative concentration of antibodies in the case of animals subjected to forced immunization.

THE EFFECT OF TRANSFUSION OF BLOOD OF DOGS IN THE LATENT PHASE OF ANTIBODY FORMATION—ARE ANTIBODIES FORMED IN THE BLOOD?

We now invite attention to Table 4 which gives the results of estimations of antibodies in the blood of dogs transfused from dogs at

TABLE 4.

ANTIBODIES IN THE SERUM OF DOGS TRANSFUSED WITH IMMUNE BLOOD AT VARIOUS PERIODS—3, 6, AND 24 HOURS, AND 2, 4, AND 6 DAYS—AFTER INJECTION OF DONORS WITH GOAT BLOOD (1 C.C. 10 PER CENT SUSPENSION PER K).

DAYS AFTER TRANSFUSION	NO. OF HOURS AND DAYS AFTER INJECTION OF DONOR WITH ANTIGEN									
	3 Hrs.	6 Hrs.	24 Hrs.	48 Hrs.	4 Days			6 Days		
	Lysin	Lysin	Lysin	Lysin	Lysin	Aggl.	Ops.	Lysin	Aggl.	Ops.
Just before transfusion	24	24	24	16	32	8	8	24	12	12
Just after transfusion	24	24	24	16	768	48	24	6,144	192	192
2	24	24	24	..	...	..	..	...	...	...
3	..	..	24	24	192	24	24	3,072	96	96
4	24	24	24	..	...	..	..	....	..	..
5	..	..	48	..	...	..	..	...	..	..
6	24	24	48	..	...	..	..	...	..	..
7	24	24	24	12	144	16	8	384	48	48
8	24	24	24	..	...	..	..	...	..	..
10	24	24	..	..	...	..	..	...	..	..
11	..	..	24	..	...	..	..	...	...	..
14	..	..	..	..	48	16	8	48	24	24

various periods after the injection of goat blood. One outstanding feature is that no antibodies appear to have been produced by or in the transfused blood itself or as a consequence of the transfusion. This is indicated especially by the absence of any increase of antibodies

[1] Braude and Carlson. "The Influence of Various Lymphagogues on the Relative Concentration of Bacterioagglutinins in Serum and Lymph," *Amer. Jour. Phys.*, 1908, 21, p. 221; Hughes and Carlson, "The Relative Hemolytic Power of Serum and Lymph under Varying Conditions of Lymph Formation," *ibid.*, p. 237; Becht and Greer, "A Study of the Concentration of the Antibodies in the Body Fluids of Normal and Immune Animals," *Jour. Infect. Dis.*, 1910, 7, p. 127.

[2] "On the Diastases in the Blood and Body Fluids," *Amer. Jour. Phys.*, 1908, 23, p. 148.

in the animals transfused from donors in an earlier stage of immunization than the fourth day. If antibodies are formed in or by the blood of immunized animals it would be reasonable to look for a production of antibodies in the receivers of the blood of dogs injected with goat blood two days and earlier before the transfusion, especially in view of the large amount of blood successfully transfused. But no increase took place.

The diminution of antibodies in the recipients of blood that contained newly formed antibodies follows the course typical of passive immunization as established by Madsen[1] and others, the decrease at first taking place rather rapidly and then more slowly, as shown well by the determination in the case of transfusion on the eighth day after the donor was injected with goat blood and when the concentration of antibodies was at its height.

Our results consequently warrant the inference that in suitable animals antigens are quickly removed from the blood or in some way so changed that the antigenic property is lost. At all events we may say that in dogs injected intravenously with 1 c.c. 10 per cent suspension of goat blood per kilo of weight, which seems to be an optimum antigenic dose in this particular case, this removal or change of antigen takes place within 3–48 hours; for there is no new formation of antibodies in dogs transfused with a large part of the blood of dogs so injected. If the blood so transfused contained free and unchanged antigen, antibodies surely would have developed in the recipient. In full concord with this result as to the recipient is the fact that the new formation of antibodies proceeds in a perfectly typical manner in donors that are transfused immediately from healthy dogs, the curve in some cases reaching a very high mark, possibly on account of the stimulus of the loss of blood on the blood-forming organs which we have reason to believe play a principal part in the formation of antibodies.

It is reasonable to assume that the power of the antibody forming cells to take up antigenic substances sooner or later reaches its limit. If that be the case the quantity of antigen in excess of this limit might remain in the circulating blood for some time after its introduction. After the injection of rabbits with 30–35 c.c. of ox blood freed from

[1] Th. Madsen, *The Decrease of Antibodies in the Organism Indicated by a Formula* (Festskrift, Statens Serum Institut, Copenhagen, 1902).

serum, Sachs[1] found by means of a specific lysin which did not lake rabbit corpuscles, that ox blood remained free in the rabbit blood for two to three days and even longer, disappearing in a more or less critical fashion as the lytic amboceptor was produced in response to the immunization. Free excess of antigen may be detected also by the method of transfusion as shown by the following experiment:

The donor was injected with 1 c.c. of goat blood per kilo of weight 14 hours previously; just before the transfusion 500 c.c. of blood were removed from the recipient; the transfusion was continued until the pressure reached normal; the 500 c.c. of blood removed from the recipient were defibrinated and infused into the donor. The following figures give the highest lytically active dilution of the serum of the recipient in the presence of 0.2 c.c. of a 5 per cent suspension of goat corpuscles and 0.012 c.c. of fresh guinea-pig serum:

Days after Transfusion	Highest Active Dilution of Serum
1	24
2	24
3	48
4	48
6	768
8	1,536
10	1,536
12	768
14	384
17	384
19	192
21	192

So far as the amount of lysin in the blood of the recipient indicates, a small quantity only of free antigen was introduced in the transfused blood. Evidently the larger part of the goat blood had been removed from the blood of the donor, and that the antibody-forming cells took part in this removal is indicated by the fact that antibody formation proceeded without disturbance, the serum on the sixth day causing lysis in a dilution of 1:36,864.

Our results consequently support the view that antibodies in active immunization are produced outside of the circulating blood into which they find their way at the end of the latent period.[2]

[1] Sachs, "Ueber die Vorgänge im Organismus bei der Transfusion fremdartigen Blutes," *Arch. f. Anat. u. Physiol.*, 1903, p. 44.

[2] V. Dungern (*Die Antikörper*, Jena, 1903) transfused normal rabbits from rabbits in the stage of latency in precipitin formation following the injection of crab plasma (Maja squinado). The transfused animals showed no sign of precipitin formation; they seemed, however, to respond to injection of crab plasma somewhat more quickly than fully normal animals, but whether because blood cells are concerned in antibody formation or for other reasons is not known.

SUMMARY.

In active immunization of dogs by a single intravenous injection of goat blood the lysin, agglutinin, and opsonin for goat corpuscles reach their highest concentration in the blood. They are uniformly slightly less concentrated in the thoracic lymph and the neck lymph, while in the cerebrospinal fluid and aqueous humor only traces of the lysin and the opsonin can be detected at the height of immunity.

In dogs similarly immunized with rat blood the opsonin and agglutinin in the blood and lymph describe parallel curves, the concentration being greatest in the blood. In the cerebrospinal fluid opsonin only is present and while the concentration is much lower than that of the blood and lymph, the curves are parallel. No increase in lysin for rat corpuscles was demonstrated by the methods used.

This relative concentration of antibodies in the blood and lymph seems to obtain in normal animals as well as in all the stages of immunization.

On transfusion of blood of dogs immunized with goat blood into normal dogs previously bled dry through the carotid the antibodies can be detected in the lymphs of the recipient in 30 minutes and in a short time the same relative distribution of the antibodies is effected as in active immunization. It seems therefore probable that in active immunization the distribution depends on the equilibrium relation between the blood and the lymph rather than on the place of formation of the antibodies, and that the rate of passage of the antibodies from the blood to the lymph is probably in part a function of the concentration in the blood.

There appears to be no difference in the rate of passage of the various antibodies for the blood to the lymph, but our methods might not disclose slight variations.

When the blood of an immune animal is transfused into a normal animal previously bled dry there is a rapid fall in the concentration of antibodies during the first 24 to 48 hours; then follows a more gradual disappearance of the antibodies until the normal limit is reached, the rate of diminution being a measure of the rate of destruction and elimination of antibodies as there is no production in the transfused blood. Hence the duration of passive immunity after as

complete transfusion as possible depends on the concentration of the antibodies in the donor's blood and the quantity of this blood transfused, a point of possible importance in connection with direct transfusion for therapeutic purposes in infectious diseases.

Bleeding a dog dry while in the latent period after intravenous injection of goat blood and then transfusing him from a normal dog may have no other than a stimulative effect on the processes of antibody formation. And transfusion of a normal dog (previously bled dry) from a dog injected intravenously with an optimum antigenic dose of goat blood from 3 to 48 hours previously, does not lead to the production of antibodies in the recipient. If the transfusion is made some time later than indicated after the introduction of the antigen the result is a simple passive immunization. These facts indicate that in this case the blood takes no direct part in the fixation of the antigen or the production of antibodies.